CLAIMS

What is claimed is:

1. A method for the identification of an analyte comprising:

(a) providing a light scanning source which produces light over an analytical wavelength range;

(b) providing at least two substantially spherical identifiable particles;

(c) applying at least one capture probe to the particles of (b) which binds to the surface of the particle, the at least one capture probe having affinity for at least one analyte;

(d) scanning each particle of (c) one or more times over a first analytical wavelength range to produce at least one first reference resonant light scattering signature for each particle of (c), said first resonant light scattering signature uniquely identifying each particle;

(e) correlating the at least one capture probe with each identified particle of (d);

(f) contacting the particle of (e) with a sample suspected of containing at least one analyte where, if the analyte is present in said sample, binding occurs between the at least one capture probe and the at least one analyte;

(g) scanning the particles of (f), one or more times over a second analytical wavelength range to produce at least one second binding resonant light scattering signature for each particle of (f), wherein:

1) the at least one first reference and at least one second binding resonant light scattering signatures may be the same or different; and

2) the at least first and second analytical wavelength ranges may be the same or different;

(h) detecting binding of the at least one analyte to the at least one capture probe by comparing the differences between the resonant light scattering signatures selected from the group consisting of: any of the at least one first reference light scattering signature and any of the at least one second light scattering signature; and

(i) identifying one or more bound analytes on the basis of the correlation made in step (e) and the at least one second binding resonant light scattering signature.

2. A method for the identification of an analyte comprising:

(a) providing a light scanning source which produces light over an analytical wavelength range;
(b) providing at least two substantially spherical identifiable particles;
(c) applying at least one capture probe to the particles of (b) which binds to the surface of the particle, the at least one capture probe having affinity for at least one analyte;
(d) affixing the particles of (c) in a defined spatial array wherein each particle has a defined locus;
(e) optionally scanning the particles of (d) one or more times over the analytical wavelength range to produce at least one first reference resonant light scattering signature for each particle of (d);
(f) contacting the particle of (e) with a sample suspected of containing at least one analyte where, if the analyte is present, binding occurs between the at least one capture probe and the at least one analyte;
(g) scanning the particles of (f) one or more times over the analytical wavelength range to produce at least one second binding resonant light scattering signature for each particle of (f);
(h) detecting binding of the at least one analyte to the at least one capture probe by comparing the differences between the resonant light scattering signatures selected from the group consisting of: any of the at least one first reference light scattering signature and any of the at least one second light scattering signature; and
(i) identifying one or more bound analytes on the basis of the affixed particle locus.

3. A method for the identification of an analyte comprising:
(a) providing light scanning source which produces light over an analytical wavelength range;
(b) providing at least two substantially spherical identifiable particles;
(c) applying at least one capture probe to the particles of (b) which binds to the surface of the particle, the at least one capture probe having affinity for at least one analyte;
(d) scanning each particle of (c) one or more times over the analytical wavelength range to produce at least one first reference

resonant light scattering signature for each particle of (c), said first resonant light scattering signature uniquely identifying each particle;
(e) correlating the at least one capture probe with each identified particle of (d);
(f) contacting the particle of (e) with a sample suspected of containing at least one analyte where, if the analyte is present, binding occurs between the at least one capture probe and the at least one analyte, the analyte comprising a detectable label; and
(g) identifying one or more analytes on the basis of the correlation of step (e) and the detectable label of the analyte.

4. A method for the detection of analyte binding to a capture probe comprising:

(a) providing a light scanning source which produces light over an analytical wavelength range;
(b) providing at least one substantially spherical identifiable particle;
(c) applying at least one capture probe to the particles of (b) which binds to the surface of the particle, the at least one capture probe having affinity for at least one analyte;
(d) optionally scanning the particles of (c) one or more times over the analytical wavelength range to produce at least one first reference resonant light scattering signature for each particle of (c);
(e) contacting the particle of (d) with a sample suspected of containing at least one analyte where, if the analyte is present, binding occurs between the at least one capture probe and the at least one analyte;
(f) scanning the particles of (e) one or more times over the analytical wavelength range to produce at least one second binding resonant light scattering signature for each particle of (e); and
(g) detecting binding of the at least one analyte to the at least one capture probe by comparing the differences between the resonant light scattering signatures selected from the group consisting of: any of the at least one first reference light scattering signature and any of the at least one second light scattering signature.

5. A method for the detection of analyte dissociation from a capture probe comprising:

(a) providing a light scanning source which produces light over an analytical wavelength range;

(b) providing at least one substantially spherical identifiable particle comprising:

1) at least one capture probe affixed to the particle and;

2) at least one analyte bound to the at least one capture probe;

(c) scanning the particle of (b) one or more times over the analytical wavelength range to produce at least one first reference resonant light scattering signature for said particle;

(d) dissociating the at least one analyte from the at least one capture probe of the particle of step (c);

(e) scanning the particle of (d) one or more times over the analytical wavelength range to produce at least one second dissociation resonant light scattering signature for each particle; and

(f) detecting dissociation of the at least one analyte from the at least one capture probe by comparing the differences between the resonant light scattering signatures selected from the group consisting of: any of the at least one first reference light scattering signature and any of the at least one second light scattering signature.

6. A method according to either of Claims 1 or 3 wherein the particle is scanned over the analytical wavelength range prior to applying the capture probe to produce an identifying resonant light scattering signature.

7. A method according to any of Claims 1-5 wherein the analytical wavelength range is a window spanning about 1 to about 20 nanometers, within optical wavelengths ranging from about 275 to about 1900 nanometers.

8. A method according to Claim 4 wherein the analyte is optionally identified by analytical methods.

9. A method according to Claim 8 wherein the analytical methods are selected from the group consisting of mass spectroscopy, fluorescence, optical absorbance radioactivity, and surface plasmon resonance.

10. A method according to Claim 8 wherein the analytical methods comprise a detectable label selected from the group consisting of fluorescent moieties, chemiluminescent moieties, particles, enzymes,

radioactive tags, quantum dots, light emitting moieties, light absorbing moieties, intercalating dyes and members of binding pairs.

11. A method according to any of Claims 1, 2 or 4 wherein the amount of bound analyte is determined by comparing the differences between the resonant light scattering signatures selected from the group consisting of: the first reference light scattering signature and any of the at least one second light scattering signature.

12. A method according to Claim 5 wherein the amount of dissociated analyte is determined by comparing the differences between the resonant light scattering signatures selected from the group consisting of: the first reference light scattering signature and any of the at least one second light scattering signature.

13. A method according to Claim 3 wherein the detectable label is selected from the group consisting of fluorescent moieties, chemiluminescent moieties, particles, enzymes, radioactive tags, light emitting moieties, light absorbing moieties, intercalating dyes and members of binding pairs.

14. A method according to Claim 7 wherein the optical wavelengths range from about 600 to about 1650 nanometers.

15. A method according to Claim 7 wherein the optical wavelengths range from about 770 to about 780 nanometers.

16. A method according to any of Claims 1-5 wherein the particle is about 100 micrometers in diameter or less.

17. A method according to any of Claims 1-5 wherein the particle is about 75 micrometers in diameter or less.

18. A method according to any of Claims 1-5 wherein the particle is about 50 **micrometers** in diameter or less.

19. A method according to any of claims 1-5 wherein the particle is substantially transparent to light over the analytical wavelength range.

20. A method according to any of Claims 1-5 wherein the particle comprises:

a) a substantially spherical core; and

b) one or more layers overlaying the core;

wherein the one or more layers is substantially transparent to light over the analytical wavelength range.

21. A method according to Claim 20 wherein the one or more layers are optically active.

22. A method according to Claim 20 wherein the one or more layers are biologically active.

23. A method according to Claim 20 wherein the one or more layers are chemically active.

24. A method according to either of Claims 22 or 23 wherein the layers have a thickness ranging from about 1 nanometer to about 10 micrometers.

25. A method according to Claim 21 wherein the core is light absorbing.

26. A method according to Claim 20 wherein the one or more layers has a thickness of about 1 nanometer to about 20 micrometers.

27. A method according to Claim 21 wherein the one or more layers have a thickness of about 50 nanometers to about 20 micrometers.

28. A method according to Claim 20 wherein the core is comprised of materials selected from the group consisting of: glasses, silica, polystyrene, polyester, polycarbonate, acrylic polymers, polyacrylamide, polyacrylonitrile, polyamide, fluoropolymers, silicone, celluloses, semiconducting materials, optically absorbing materials, metals, magnetic materials, minerals, nanoparticles, colloidal particles, metal oxides, metal sulfides, metal selenides, and composites thereof.

29. A method according to Claim 28 wherein the magnetic material is iron oxide.

30. A method according to Claim 20 wherein the core is hollow.

31. A method according to Claim 20 wherein the layers are comprised of materials independently selected from the group consisting of: glasses, silica, polystyrene, polyester, polycarbonate, acrylic polymers, polyacrylamide, polyacrylonitrile, polyamide, fluoropolymers, silicone, celluloses, polyelectrolytes, minerals, nanoparticles, colloidal particles, metal oxides, metal sulfides, metal selenides, and composites thereof.

32. A method according to any of Claims 1-5 wherein the particle is comprised of glass having an index of refraction of about 1.45 to about 2.1 over the analytical wavelength range.

33. A method according to any one of Claims 1-5 wherein the at least one capture probe is selected from the group consisting of proteins, nucleic acids, peptide nucleic acids, one member of a binding pair, antibodies, biological cells, microorganisms, cell membrane fragments,

cellular organelles, receptors, viruses, viral fragments, bacteriophage, bacteriophage fragments, organic ligands, and organometallic ligands.

34. A method according to Claim 33 wherein the at least one analyte is present in a sample comprising sample matrix components.

35. A method according to any one of Claims 1-5 wherein the at least one analyte is selected from the group consisting of proteins, nucleic acids, peptide nucleic acids, biological cells, microorganisms, cell membrane fragments, cellular organelles, antibodies, receptors, viruses, viral fragments, bacteriophage, bacteriophage fragments, and one member of a binding pair.

36. A method according to Claim 10 wherein the one member of a binding pair is selected from the binding pair combinations consisting of: antigen/antibody, antigen/antibody fragment, Protein A/antibody, Protein G/antibody, hapten/anti-hapten, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein; hormone/hormone receptor, lectin/carbohydrate, enzyme/enzyme cofactor, enzyme/substrate, enzyme/inhibitor, peptide nucleic acid/complimentary nucleic acid, polynucleotide/polynucleotide binding protein, vitamin B12/intrinsic factor; complementary nucleic acid segments; pairs comprising sulfhydryl reactive groups, pairs comprising carbodiimide reactive groups, and pairs comprising amine reactive groups.

37. A method according to Claim 33 wherein the one member of a binding pair is selected from the binding pair combinations consisting of: antigen/antibody, antigen/antibody fragment, Protein A/antibody, Protein G/antibody, hapten/anti-hapten, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein; hormone/hormone receptor, lectin/carbohydrate, enzyme/enzyme cofactor, enzyme/substrate, enzyme/inhibitor, peptide nucleic acid/complimentary nucleic acid, polynucleotide/polynucleotide binding protein, vitamin B12/intrinsic factor; complementary nucleic acid segments; pairs comprising sulfhydryl reactive groups, pairs comprising carbodiimide reactive groups, and pairs comprising amine reactive groups.

38. A method according to Claim 35 wherein the one member of a binding pair is selected from the binding pair combinations consisting of: antigen/antibody, antigen/antibody fragment, Protein A/antibody, Protein G/antibody, hapten/anti-hapten, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein; hormone/hormone receptor,

lectin/carbohydrate, enzyme/enzyme cofactor, enzyme/substrate, enzyme/inhibitor, peptide nucleic acid/complimentary nucleic acid, polynucleotide/polynucleotide binding protein, vitamin B12/intrinsic factor; complementary nucleic acid segments; pairs comprising sulfhydryl reactive groups, pairs comprising carbodiimide reactive groups, and pairs comprising amine reactive groups.

39. A method according to any one of Claims 1-5 wherein the capture probe is synthesized on the surface of the particle.

40. A method according to any one of Claims 1-5 wherein the capture probe is isolated from natural sources or synthesized separately prior to being added to the surface of the particle.

41. A method according to Claim 34 wherein after applying the capture probe to the particle, the particle is treated to prevent non-specific binding of sample matrix components.

42. A method according to Claim 34 wherein the particles are coated with a thin film to prevent non-specific binding of components of the sample matrix and then the thin film coating is activated to attach the capture probe.

43. A method according to any one of Claims 1, and 3 wherein the either the first reference or second binding resonant light scattering signature comprises spectral features selected from the group consisting of; peak wavelength positions, peak widths, wavelength intervals among peaks, peak amplitudes, and polarization-dependent properties.

44. A method according to any one of Claims 1, 2, 4 and 5 wherein the reference and binding resonant light scattering signatures are compared on the basis of spectral features selected form the group consisting of; peak wavelength positions, peak widths, wavelength intervals among peaks, peak amplitudes, and polarization-dependent properties.

45. A method according to any of Claims 1-5 wherein the particle comprises one of more optically active layers.

46. A method according to any of Claims 1-5 wherein the particle comprises one of more biologically active layers.

47. A method according to any of Claims 1-5 wherein the particle comprises one of more chemically active layers.

48. An identifiable particle comprising:

(a) a substantially spherical core;

(b) a capture probe affixed to the outer surface of the particle;

wherein:

1) the particle is characterized by a unique resonant light scattering signature when scanned over an analytical wavelength range of about 1 to about 20 nanometers over a range of optical wavelengths of about 275 nanometers to about 1900 nanometers;

2) the particle is about 100 micrometers in diameter or less;

3) the particle has a refractive index between about 1.6 and about 2.1 over the analytical wavelength range; and

4) the particle is substantially non-fluorescing over the analytical wavelength range.

49. A particle according to Claim 48 optionally having one or more layers overlaying the core.

50. A particle according to Claim 49 wherein the one or more layers are optically active.

51. A particle according to Claim 49 wherein the one or more layers are biologically active.

52. A particle according to Claim 49 wherein the one or more layers are chemically active.

53. A particle according to either of Claims 51 or 52 wherein the layers have a thickness ranging from about 1 nanometer to about 10 micrometers.

54. A particle according to Claim 49 wherein the one or more layers have a thickness of about 50 nanometers to about 20 micrometers.

55. An identifiable particle comprising:

(a) a substantially spherical core;

(b) a capture probe affixed to the outer surface of the particle;

wherein:

1) the particle is characterized by a unique resonant light scattering signature when scanned over an analytical wavelength range of about 1 to about 20 nanometers

over a range of optical wavelengths of about 275 nanometers to about 1900 nanometers;

2) the particle is about 100 micrometers in diameter or less;

3) the particle has a refractive index between about 1.6 and about 2.1 over the analytical wavelength range; and

4) the particle is substantially non-fluorescing over the analytical wavelength range;

wherein the particle comprises:

i) one or more optically active layers having a thickness between about 50 nanometers and about 20 micrometers; and

ii) one or more biologically active or chemically active substantially transparent outer layers of thickness between about 1 nanometer to 10 micrometers, said layers overlaying the layer of (i).

56. A particle according to Claim 48 wherein the core is comprised of materials selected from the group consisting of; glasses, semiconducting materials, optically absorbing materials, metals, magnetic materials, and composites thereof.

57. A particle according to Claim 48 wherein the capture probe is selected from the group consisting of proteins, nucleic acids, peptide nucleic acids, one member of a binding pair, antibodies, biological cells, microorganisms, cell membrane fragments, cellular organelles, receptors, viruses, viral fragments, bacteriophage, bacteriophage fragments, organic ligands, and organometallic ligands.

58. A particle according to Claim 57 wherein the one member of a binding pair is selected from the binding pair combinations consisting of: antigen/antibody; Protein A/antibody, Protein G/antibody, hapten/anti-hapten, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein; hormone/hormone receptor, lectin/carbohydrate, enzyme/enzyme cofactor, enzyme/substrate, enzyme/inhibitor, peptide nucleic acid/complimentary nucleic acid, polynucleotide/polynucleotide binding protein, vitamin B12/intrinsic factor; complementary nucleic acid

segments; pairs comprising sulfhydryl reactive groups, pairs comprising carbodiimide reactive groups, and pairs comprising amine reactive groups.

59. A population of identifiable particles according to Claim 48.

60. A microparticle based measuring system comprising:

(a) at least one substantially spherical identifiable particle in solution, each particle comprising;

(a) a capture probe affixed to the outer surface of the particle;

wherein:

1) the particle is characterized by a unique resonant light scattering signature when scanned over an analytical wavelength range having a window spanning about 1 to about 20 nanometers, over a range of optical wavelengths from about 275 to about 1900 nanometers;

2) the particle is about 75 micrometers in diameter or less; and

3) the particle has a refractive index between about 1.45 and about 2.1 over the analytical wavelength range.

(b) a light scanning source for scanning the particle over the analytical wavelength range;

(c) an optical cell for presenting the particle in a suitable position and in a suitable environment for detecting scattered light;

(d) a particle handling means for placing particles into the optical cell; and

(e) a detection means for detecting light from the scanned particle and converting said light to an electrical signal.

61. A system of Claim 60 optionally comprising a means for contacting the particles with reagents and analytes.

62. A system of Claim 60 optionally comprising a computer operably linked to the light scanning source and the detection means for the acquisition and analysis of said electrical signal generated by said detection means.

63. A system of Claim 60 optionally comprising a data analysis means for converting said electrical signal to the identity of the particle and optionally the presence or degree of binding of an analyte or group of analytes to the particle.

64. A system of Claim 60 optionally having a data analysis means for detecting binding of or identifying said analyte or group of analytes.

65. A system of Claim 60 wherein the scanned light is generated by a source selected from the group consisting of a scanning diode laser, a tunable diode laser, and a polychromatic light source.

66. A system of Claim 65 wherein the scanned light source is used with a wavelength-selecting device.

67. A system of Claim 60 wherein the scanned light source is modified with a fiber optic light coupling means.

68. A system of Claim 66 wherein said wavelength-selecting device optionally comprises components selected from the group consisting of; a dispersive element, a non-dispersive element, a monochromator, a tunable filter, a prism, a grating, and a fixed filter.

69. A system of Claim 60 wherein the scattered light from one or more particles is detected with an imaging means.

70. A microparticle based measuring system comprising according to Claim 66 wherein the imaging means comprises:

(a) a scanning diode laser light source;

(b) an optical cell suitable for spectroscopic scattered light imaging and stray light rejection;

(c) a means for contacting microparticles with analytes and reagents;

(d) a microscope;

(e) a digital camera and monitor;

(f) digital image acquisition hardware;

(g) a computer operably linked to the elements of (a) – (f) as needed and

(h) software suitable for controlling the elements of (a) – (f), capturing data, and processing the data.